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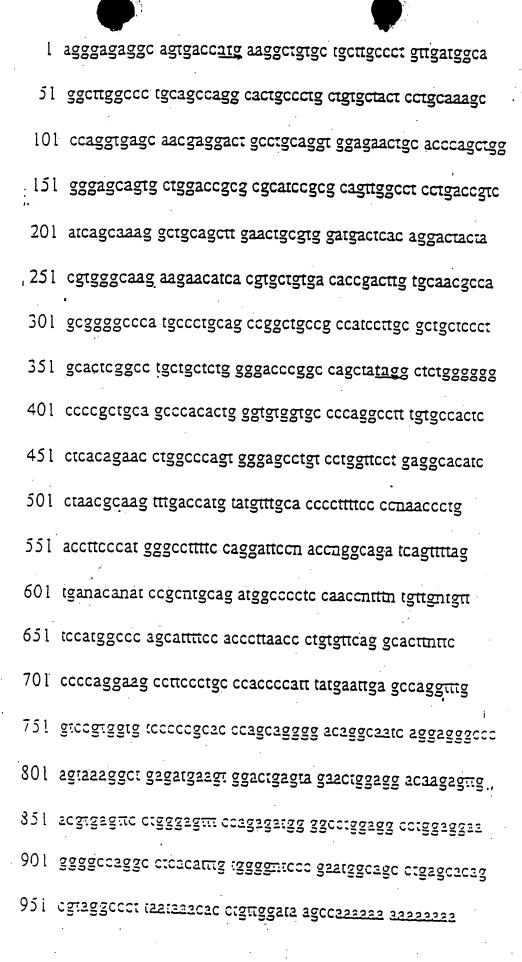
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M.A.LLALLMAGLALQPGTALLCYSCKAQVSNEDCLQNENCTQLGEQCWTARIRAVGLLTV I SKGCSLNCVDDS
ODYVVGKKNITCCDTDLCNASGAHALQPAAAILALLPAL

FIGURE 1B

	1	ATGAAGACAGTTTTTTTTATCCTGCTGGCCACCTACTTAGCCCTGCATCCAGGTGCTGCT															т					
, •		TA	CT1	CTC	TC	\AA/	VAA.	AAT	AGG	ACG	ACC	GGT	GGA	TGA	ATC	GGG	ACG	TAG	GTC	CAC	ACG	- + 60 A
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121	1	CT	GCA	GTC	CTA	TTC	ATO	CA	CAG	CAC	AGA	TGA	ACA	ACA	GAG.	ACT	GTC	rga.	\TG1	'ACA	GAA	2
		GA(	CGT	CAC	GAT	`AAG	TAC	CGT	GTC	GTG'	ICT.	ACT	rgt	rg T	CTC	TGA	CAG	CTI	`ACA	TGT	CTTC	120
		Ľ	Q	_	Y					Q							L		v	Q	N <sub>.</sub>	_
	1	TGCAGCCTGGACCAGCACAGTTGCTTTACATCGCGCATCCGGGCCATTGGACTCGTGACA ACGTCGGACCTGGTCGTGTCAACGAAATGTAGCGCGTAGCCCGGTAACCTGAGCACTGT																				
				GGA	CCT	GGT	CGT	GTC	ZAAC	GAA	ATO	TAC	CGC	GTA	GGC	CCC	GTA	ACC	TGA	GCA	CTGT	180
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181	\ L	GTI	AT	CAG	TAA( - + -	GGG	CTG 	CAG	CTC	ACA	GTO	TGA +	GGA	TGA	CTC	GGA	GAA	CTA	CTA'	TTT	GGC	_
	(	CAA	TA(	STC				GTC	GAG	TGT	CAC	ACT	CCT	ACT	GAG	CCI	CTT	GAT	GAT	AAA	ccc	240
241			I		K	_	_	S		Q		Ε	D	D	S	Ε		Y				-
		AAGAAGAACATCACGTGCTGCTACTCTGACCTGTGCAATGTCAACGGGGCCCACACCCTG TTCTTCTTGTAGTGCACGACGATGAGACTGGACACGTTACAGTTGCCCCGGGTGTGGGAC																				
	1	rrc	TTC								ACT	GGA	CAC	GTT	ACA	GTT	GCC	CCG	GTO	TGO	GAC	300
01			K		I				. <b>Y</b>		D	L	_	N	V	N	G	A	н	T	L	-
	·	AAGCCACCCACCCTGGGGCTGCTGACCGTGCTCTGCAGCCTGTTGCTGTGGGGCTCC															360					
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															S	L	L				s	-
61	T	GCCGTCTGTAGGCTCTGGGAGAGCCTACCATAGCCCGATTGTGAAGGGATGAGCTGCAC  CGGCAGACATCCGAGACCCTCTCGGATGGTATCGGGCTAACACTTCCCTACTCGACGTG															420					
	s			L	*	COA	GAC			.GGA	VTGC	TAT	CGG	GCT	`AAC	AC1	TCC	CTA	CTC	GAC	GTG	120
21	_	•	•	_		CCA	C A C	àcc														
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		-					- 4 - 3															

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| MKAVLLAULMAGEALOFGTA
| MKAVLLAULMATYUALMOFGTA
| MKIVLFLLUATYUALMOFGTA
| MKIVLFLUATYUALMOFGTA
| MKIVLFLUATYUALMOFGTA
| LCSCCCACACOVSN°EDCULOVEN
| LCCCACACOVSN°EDCULOVEN
| LCCCACACOVSN°EDCULOVEN
| MKIVLGCACACOVSN°EDCULOVEN
| MKIVLGCACACO
| MKIVLGC
```

hSCA-2 hPSCA mPSCA

\* William work to the 1014 Sirlare Nove. HEDDE Medibility Jacoson Hall igenic (nows) Alpho Helices A THE SHAPE GOR Alpha Helica. con new Wheels Glumonyl, Sites

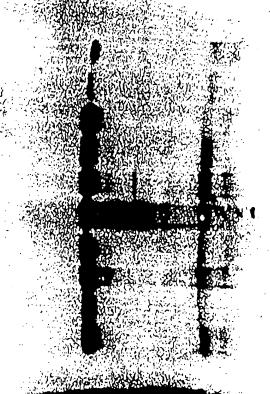
5.grale refilence

/ = 9/4cosylation

La prisigna

FIGURE 5

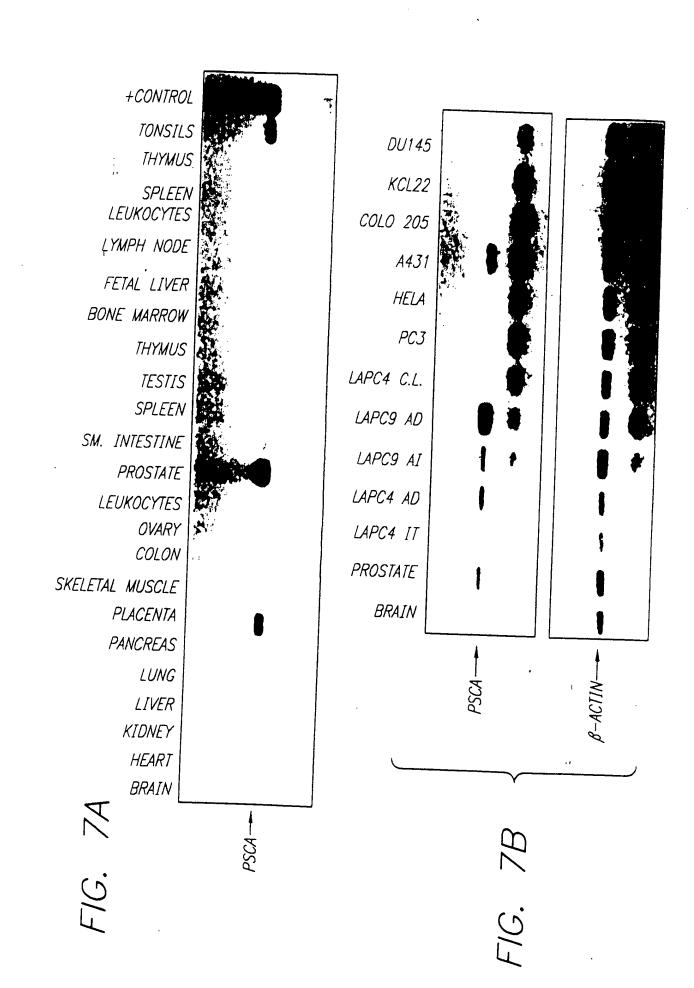
2\*

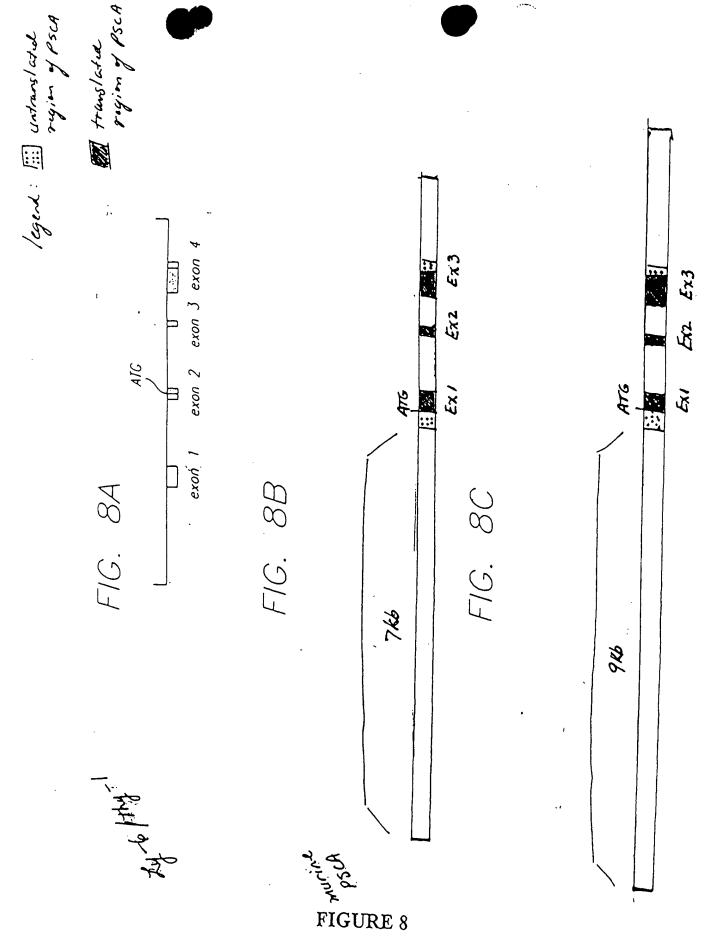


prostate (Buth)
prostate (Buth)
prostate (Buth)
Bladder (Hunter)
Bladder (dek)
Bladder (Rod)
Kidney (NLAO4)
Kedney (WU2)
Tosto
Sm. Intest.

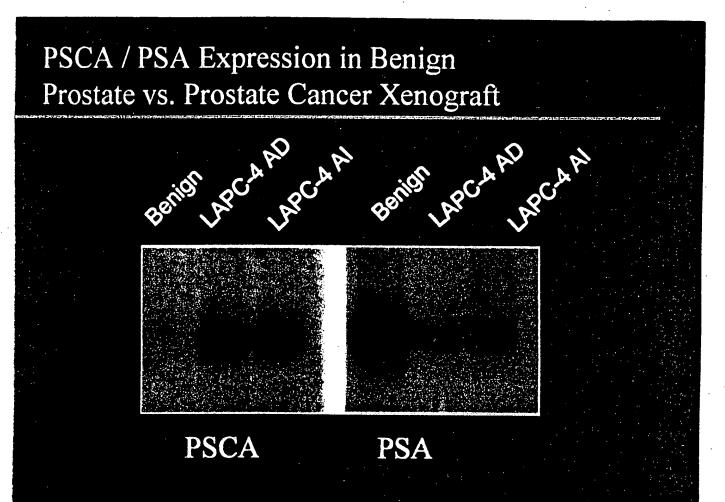
LAPCI

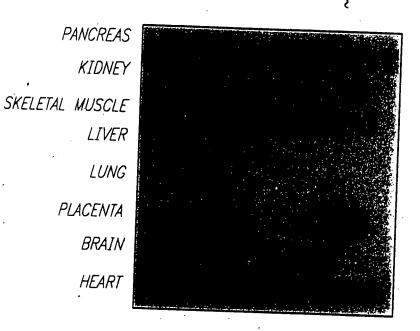
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Manage A. C.





PERIPHERAL LEUKOCYTES

COLON

SMALL INTESTINE

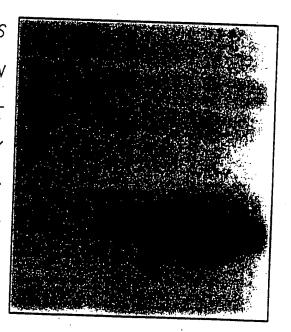
OVARY

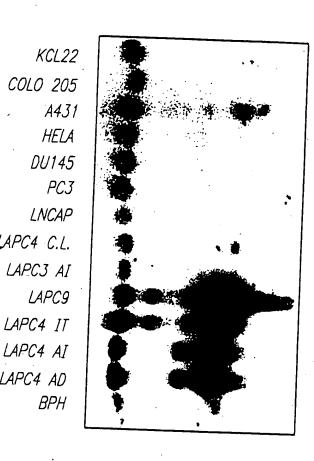
TESTIS

PROSTATE

THYMUS

SPLEEN





COLO 205
A431
HELA
DU145
PC3
LNCAP
LAPC4 C.L.
LAPC3 AI
LAPC9
LAPC4 IT
LAPC4 AD
BPH

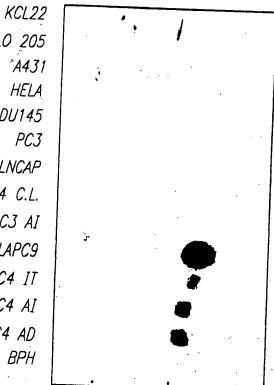
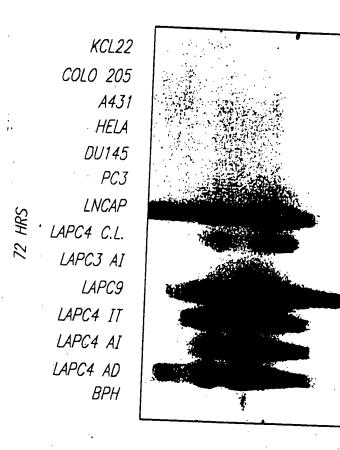


FIG. 10-1



COLO 205

A431

HELA

DU145

PC3

LNCAP

LAPC4 C.L.

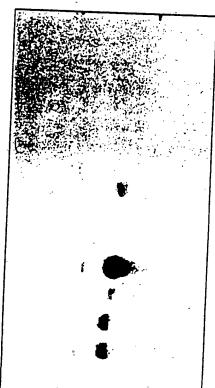
LAPC3 AI

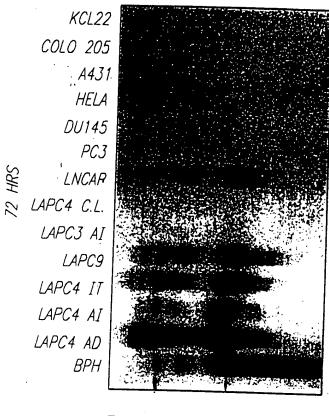
LAPC9

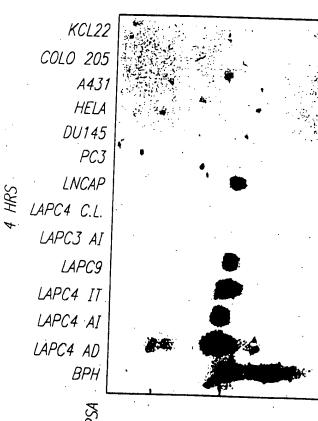
LAPC4 II

LAPC4 AD

BPH







ETBR

## FIG. 11A





FIG. 11B

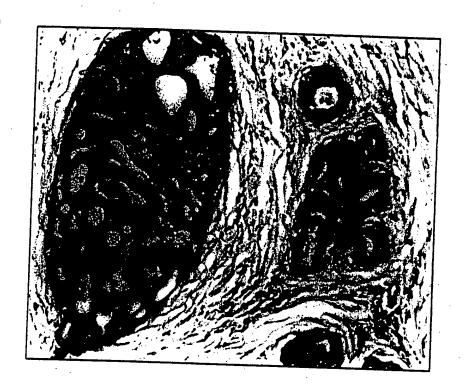


FIG. 11C

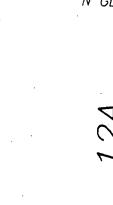


FIG. 12B

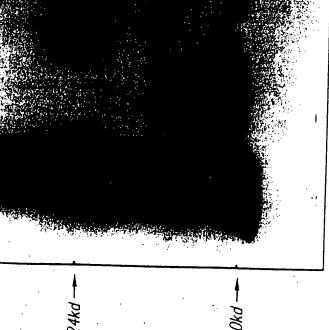
O GLYCOSIDASE

SECRETED

CELL ASSOCIATED

N GLYCOSIDASE F

CONTROL



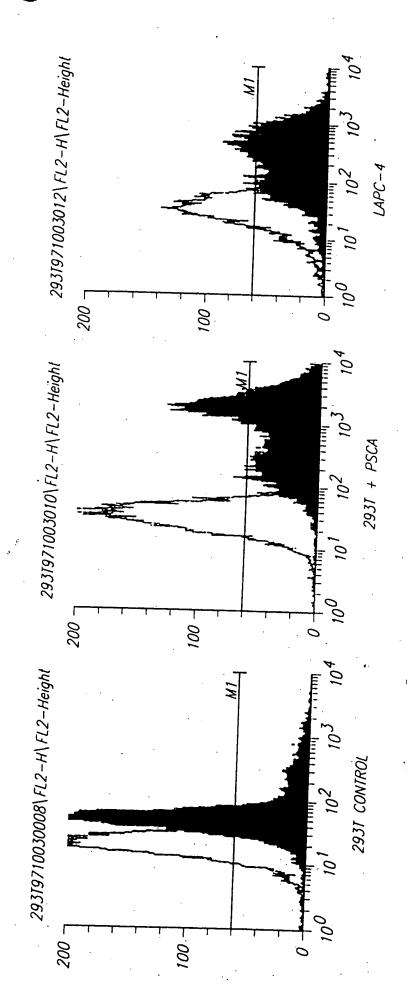
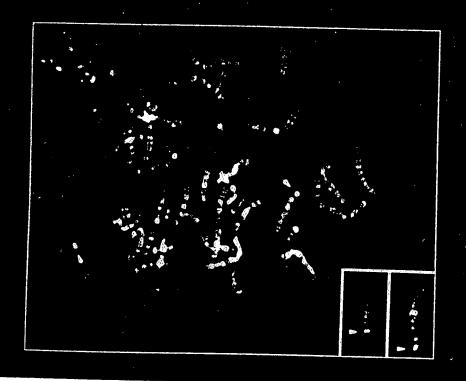
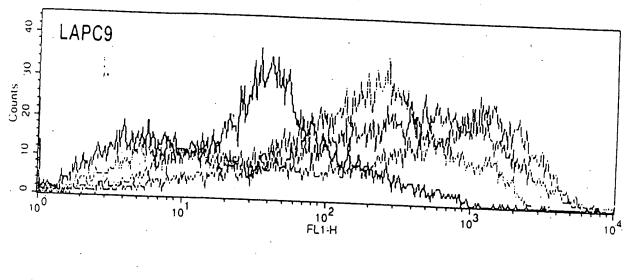


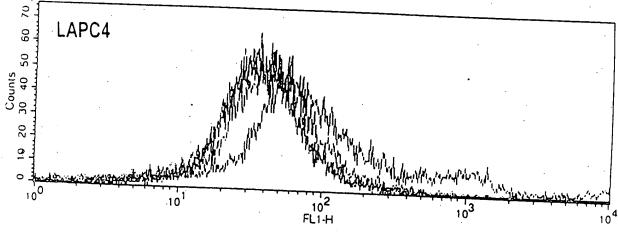
FIGURE 12C

# PSCA Maps to Chromosome 8q24.2



Fluorescent in Situ Hybridization Analysis of PSCA





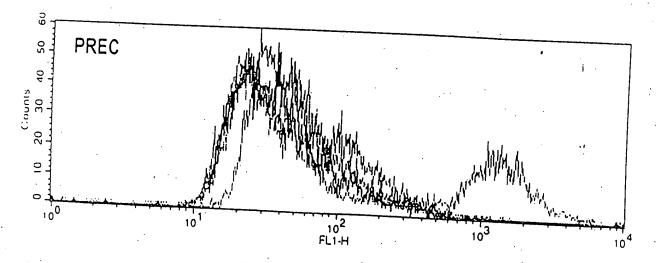


FIGURE 14

C (85-123) 0.000 0.021 0.005 0.370 0.003 0.000
M (46-109) 0.628 0.032 0.016 0.069 0.000 0.004
N (2-50) 0.007 0.863 1.965 0.024 1.315 0.733
EL (18-98) 2.039 1.318 2.893 0.328 2.039 1.366 2.805
Isotype       IgG1     k       IgG2a     k       IgG3     k       IgG2a     k       IgG2a     k       IgG2a     k       IgG2a     k
mAb 1G8 2H9 3C5 3E6 4A10 2A2 3G3

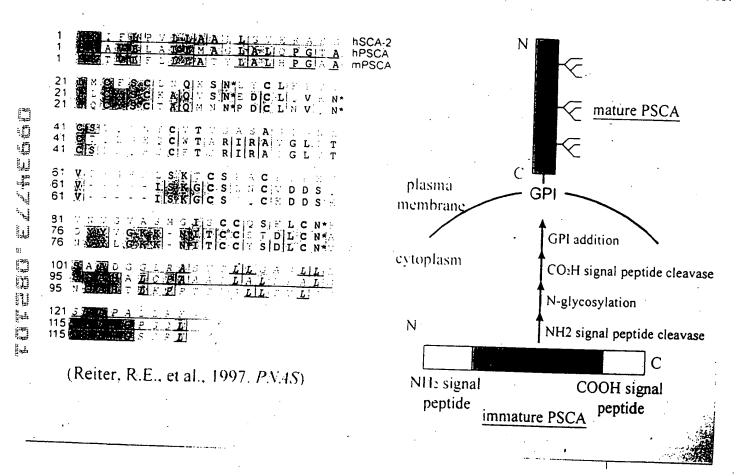
3C5 2H9 **2A2** 168



O M N L 3G3

FIGURE 15

# Prostate Stem Cell Antigen (PSCA) is a GPI-anchored Protein



# FISH Analysis of PSCA and c-myc in Prostate Cancer

#34 c-myc #75 c-myc #75 PSCA

R. Jenkins

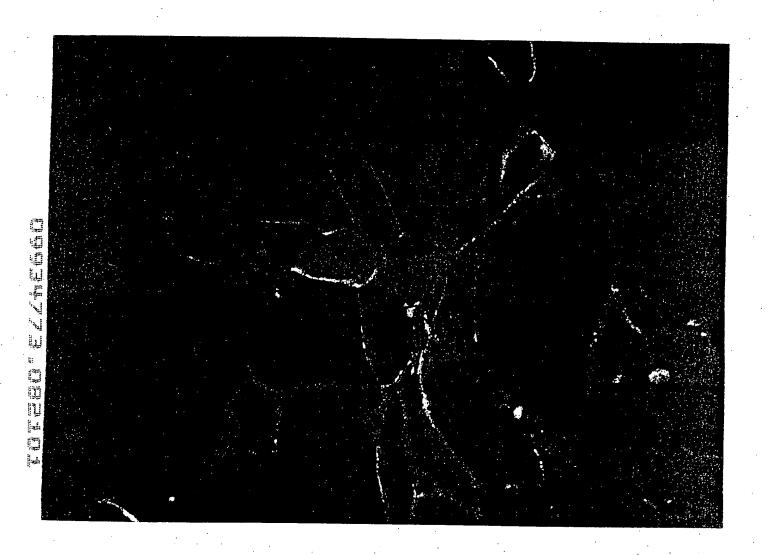


FIGURE 18

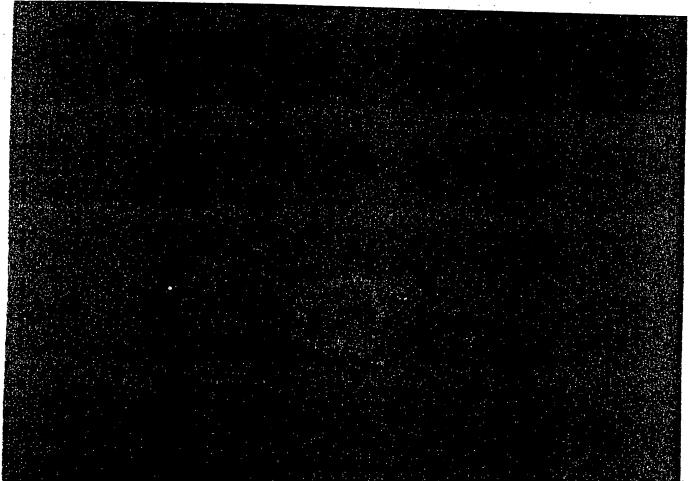


FIGURE 19



FIGURE 20

## **PSCA Immunostaining of Primary Tumors**

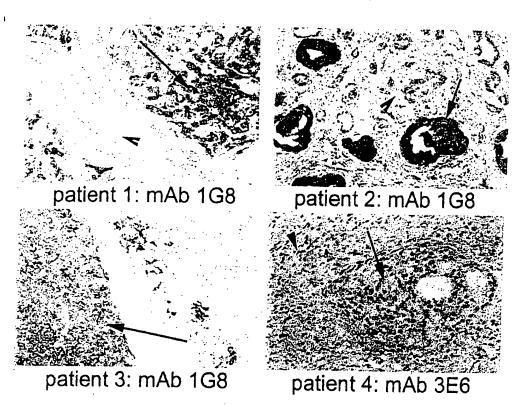




FIGURE 22

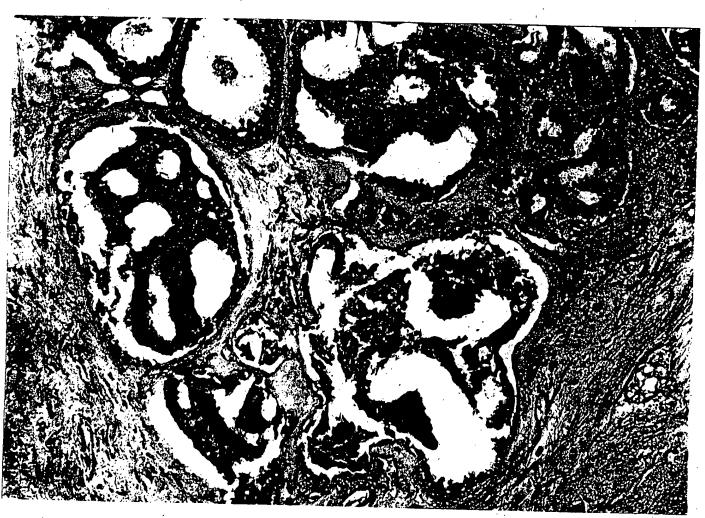


FIGURE 23

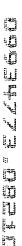
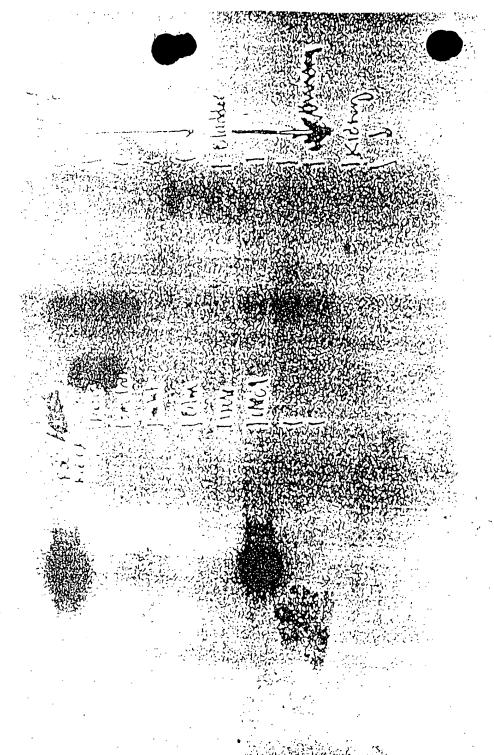




FIGURE 24



750 14nh Ch

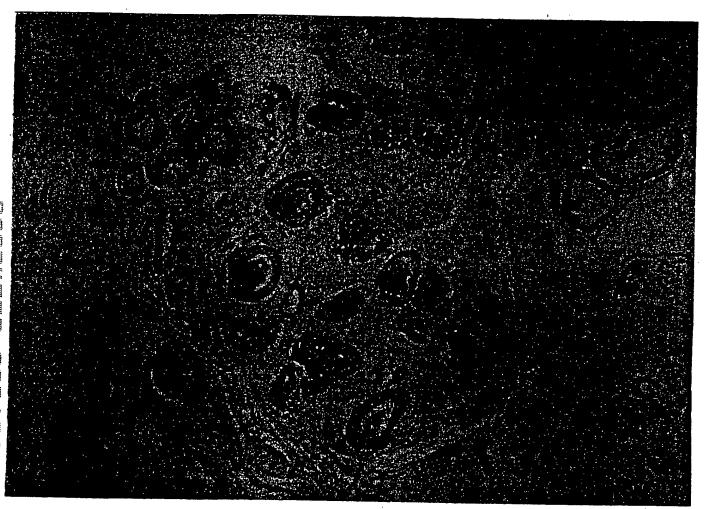


FIGURE 26

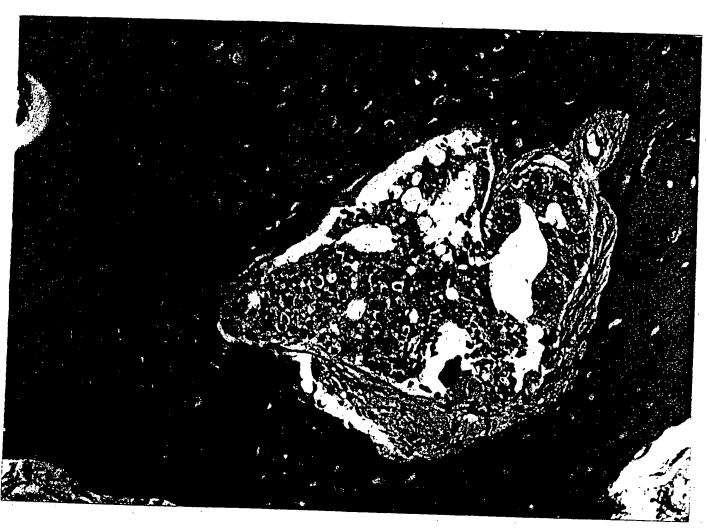
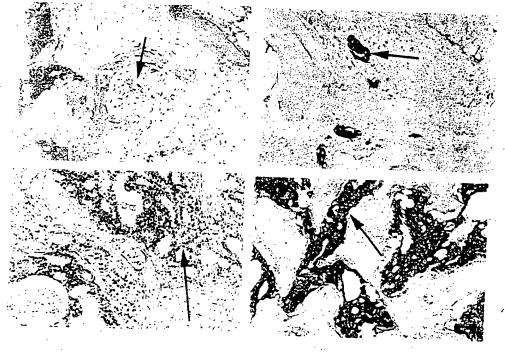


FIGURE 27

## PSCA Immunostaining of Bony Metastases



Patient 5: H and E and mAb 1G8

Patient 4: H and E and mAb 3E6



FIGURE 29

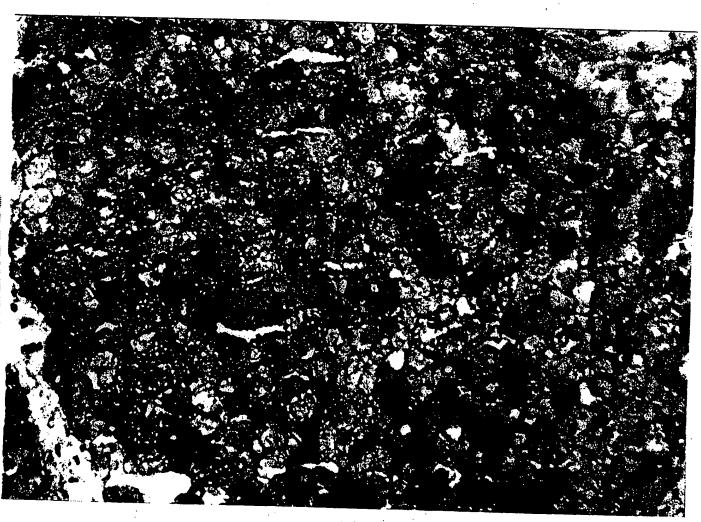


FIGURE 30

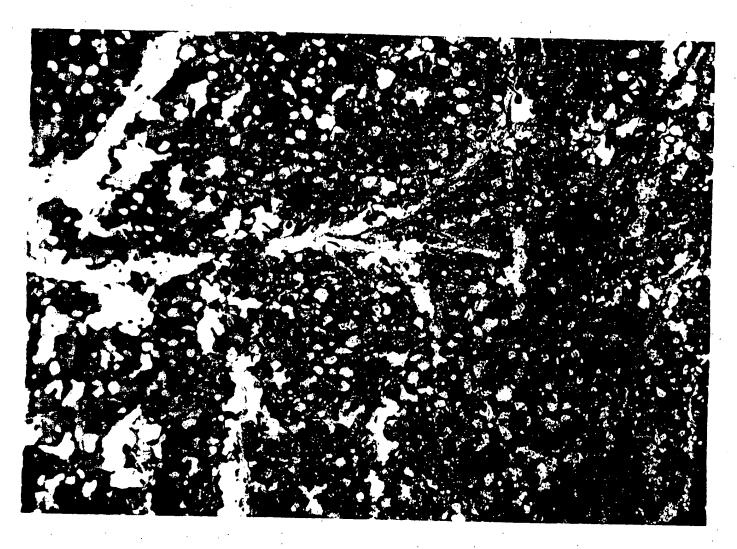


FIGURE 31

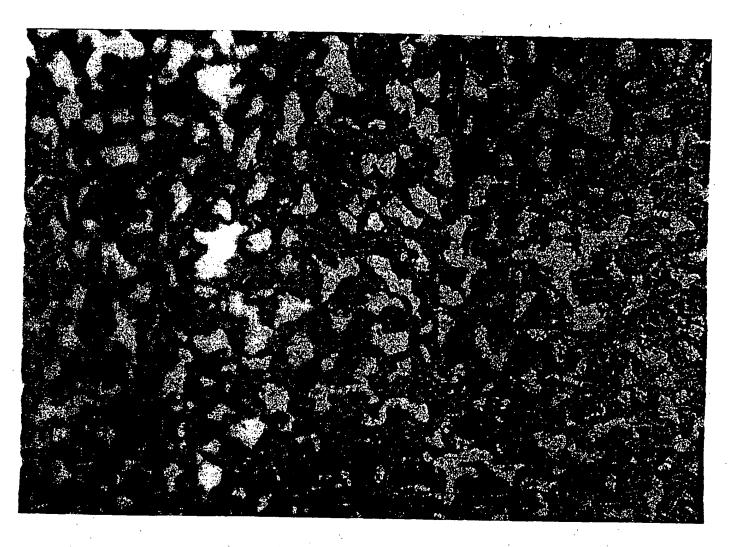


FIGURE 32

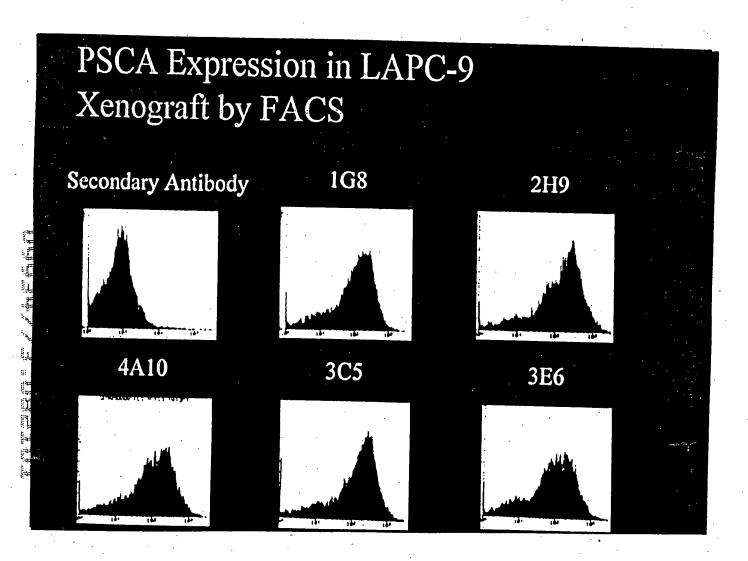


FIGURE 33

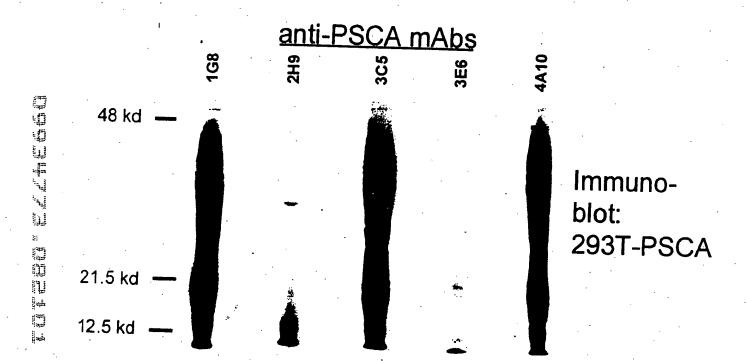


FIGURE 34

### Immunofluorescent Staining of LNCaP-PSCA Cells

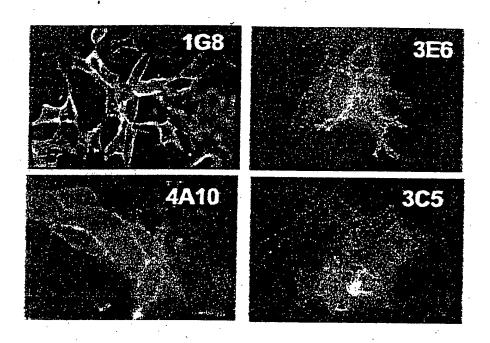


FIGURE 35

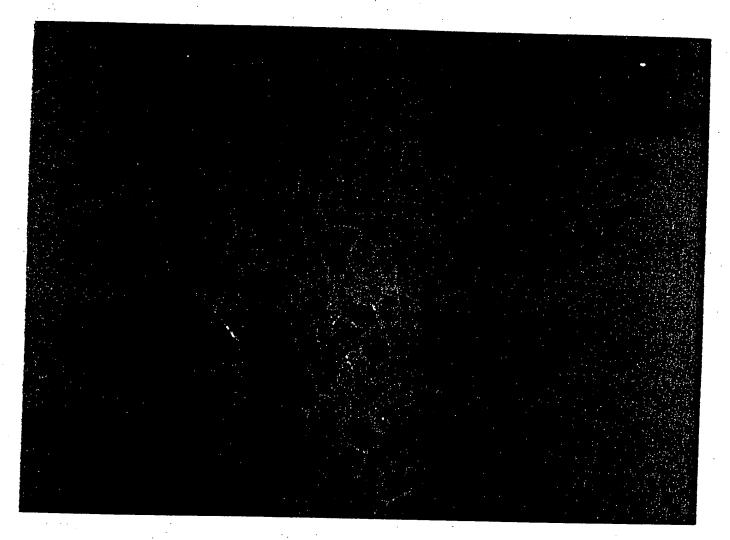
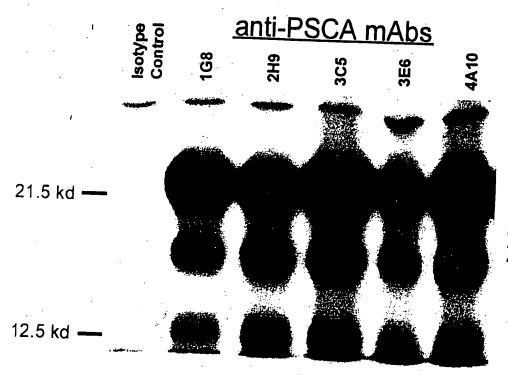


FIGURE 36



Immunoprecipitation: 293T-PSCA

FIGURE 37

### Immunohistochemical Staining of Normal Prostate

Normal: Isotype Control



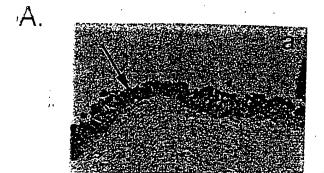
Normal: PSCA mAb 3E6



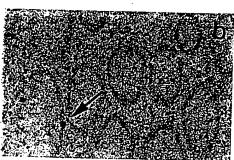


Normal: PSCA mAb 1G8 Atrophy: PSCA mAb 2H9

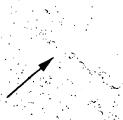




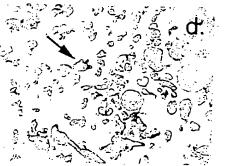
Bladder: 1G8



Colon: 1G8

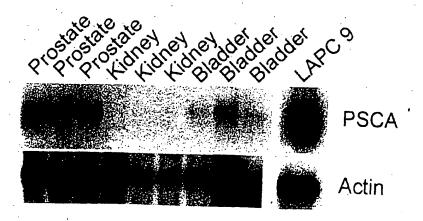


Kidney: 3E6

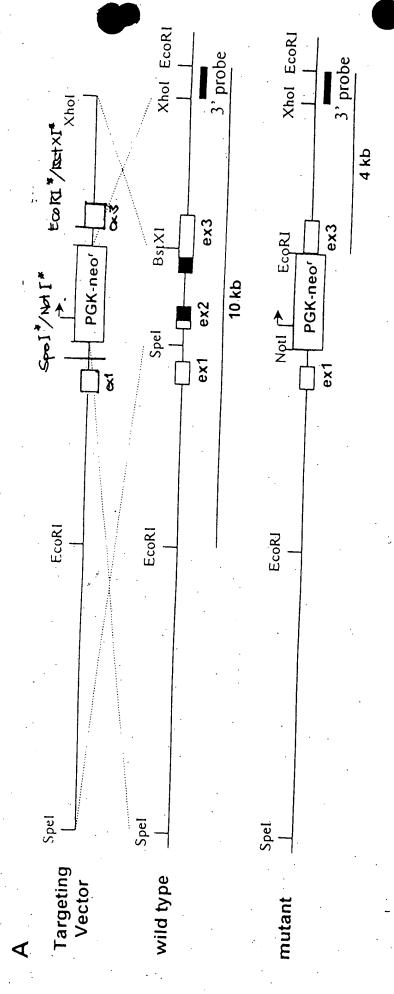


Placenta: 3E6

B.



## Targeting of Mouse PSCA Gene



\* ex1, 2, and 3 are the exons of PSCA gene.

\* Black boxes of ex2 and ex3 encode PSCA mature protein sequences.

\* ES genomic DNA's were digested with EcoRI, followed by Southern hybridization using 3' probe

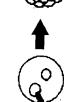
B. Genomic Southern Analysis of ES Cells
+/+ +/10 kb →
4 kb →

FIGURE 40

# Transgenic Mouse Models of Prostate Cancer

prostate
specific
promoter concogen











Mouse bearing prostate cancer

Offspring expressing oncogene in prostate

Embryo

of transgenes into

Microinjection

male pronucleus

### Target tissues

prostate (secretory cells) urethral, mammary and sweat gland

prostate (secretory cells)

Characteristics
Low-grade PIN 8-12 wks
High-grade PIN 8-12 wks
Invasive carcicinoma 28 wks
No metastases

Low-grade PIN 5-8 wks High-grade PIN 8-12 wks Invasive carcicinoma 12 wks Metastases in lymph node, lung, liver and bone

Low-grade PIN 8-12 wks High-grade PIN 8-12 wks Invasive carcicinoma 16 wks Metastases in lymph node, lung, liver and bone

Transgene

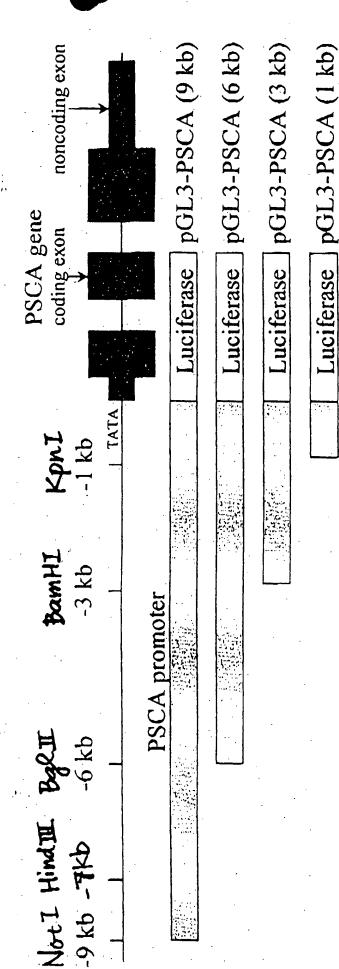
C3(1) (-3 kb)/ SV40 large+small<sub>1</sub> T Maroulakou et al. 1994 PNAS Probasin (-426 bp)/ SV40 large+small T Greenberg et al. 1995 PNAS

Cryptdin2 (-6.5 kb)/ SV40 large+small, T Garabedian et al. 1998 PNAS

prostate
(neuroendocrine cells)
small intestine

FLEURE 41

# Reporter Gene Constructs for Transfection Assay



CMV promoter

| pGL3-basic

Luciferase

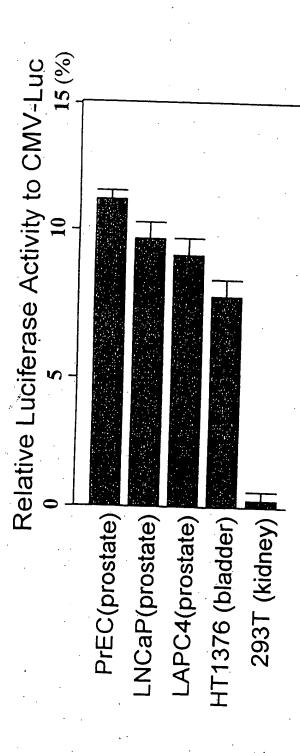


FIGURE 43

### Identification of Prostate-Specific Elements Within PSCA Promoter Sequences

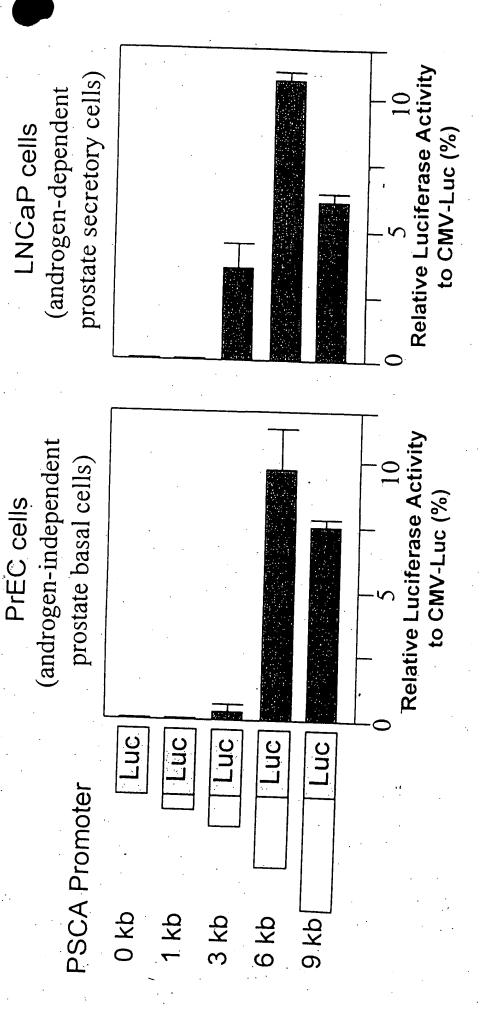


FIGURE 44

## Update of Transgenic Mouse Projects

(DNA positive) Number of Founders Genomic Structure of PSCA Exon 1 exon 2 exon 3 3'hGH intron PSCA promoter (6kb) CER | PSCA promoter (6kb) (GBI) [DA] ATG PSCA promoter (6kb) PSCA promoter (9kb) PSCA promoter (9kb) PSCA promoter (9kb) PSCA promoter PSCA(9 kb)-GFP-3'hGH PSCA(6 kb)-GFP-3'hGH PSCA(9 kb)-SV40TAG PSCA(6 kb)-SV40TAG PSCA(6 kb)-GFP PSCA(9 kb)-GFP

FIGURE 45

Negative tissues Seminal Vesicle Small intestine Stomach Urethra Colon

Prostate

Kidney **Testis** Liver

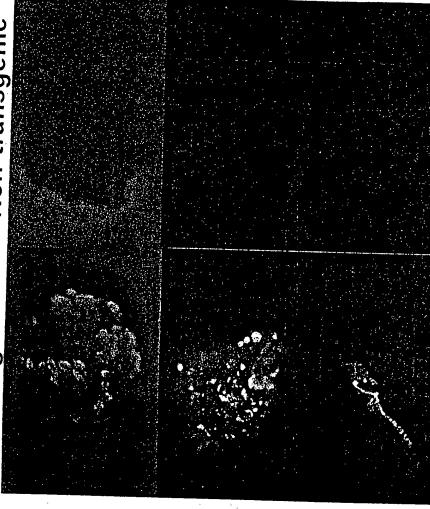
Lung Brain Heart

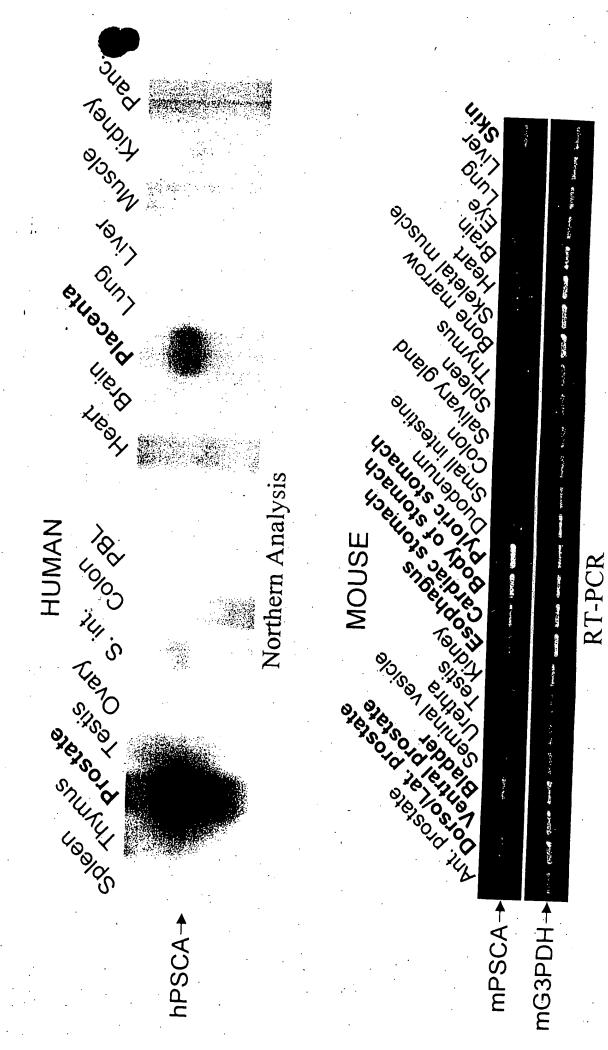
Skeletal muscle Ovary

(A25-106-2)(A25-104)Bladder

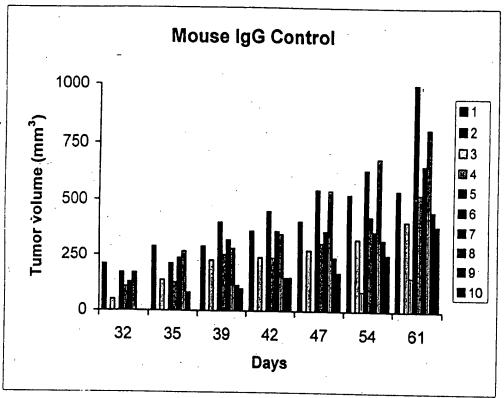
(A25-106-2)Skin

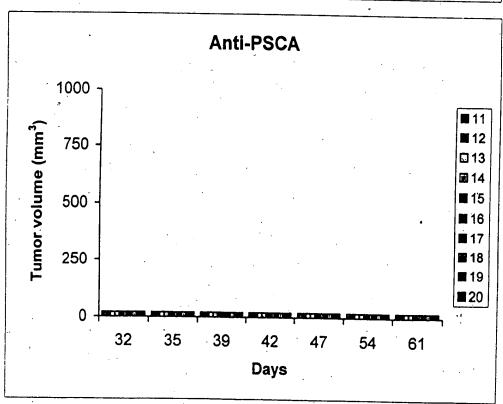
Whole-mount green fluorescence image Non-transgenic Transgenic





MGUIRE 47

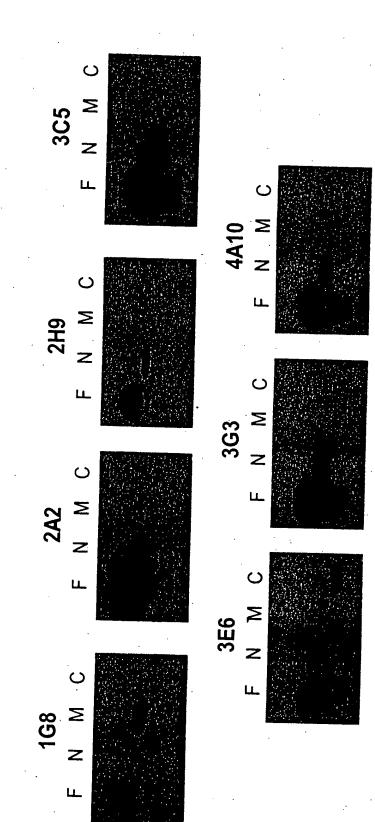




### FIG. 49

### Epitope recognized (Of

		<i>i</i> .					٠	
lm)	C (85-123)	0.003	0.010	0.001	0.002	2.118	0.000	0.001
liope recognized (OD 450 nm)	M (46-109)	1.273	0.023	0.002	900.0	1.133	0.004	0.000
Epitope recog	N (2-50)	0.004	0.631	1.026	1.709	0.036	1.731	0.493
	F (18-98)	1.485	0.973	1.069	1.916	1.609	2.805	1.053
	Sotype	lgG1 k	lgGZa k	lgG1 k	lgG2a k	1gG3	lg52a k	lgGZa K
	mAb	85.	247 21.10	2H3	5.55 5.75 5.75	350 200	3G3	- OI V

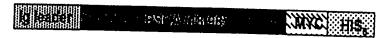


Standardization Ag

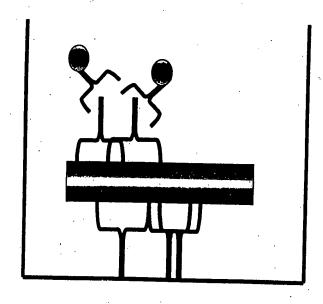
APASCA APASCA

anti-peptide
1G8 polyclonal

Engineered mammalian secreted form



B

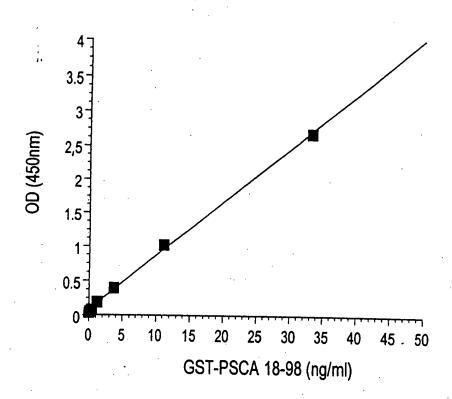


Anti-IgG2a HRP

Anti-PSCA mAbs 3C5+4A10+2A2 (IgG2a)

PSCA .

Affinity purified anti-peptide polyclonal + mAb 1G8 (lgG1)



B

<u>Sample</u>	OD+range (n=2)	<u>ng/ml</u>
vector	0.005+0.001	ND
vector+hu serum	0.004+0.001	ND'
secPSCA	2.695+0.031	32.92
secPSCA+hu serum	2.187+0.029	26.55

FIG. 52

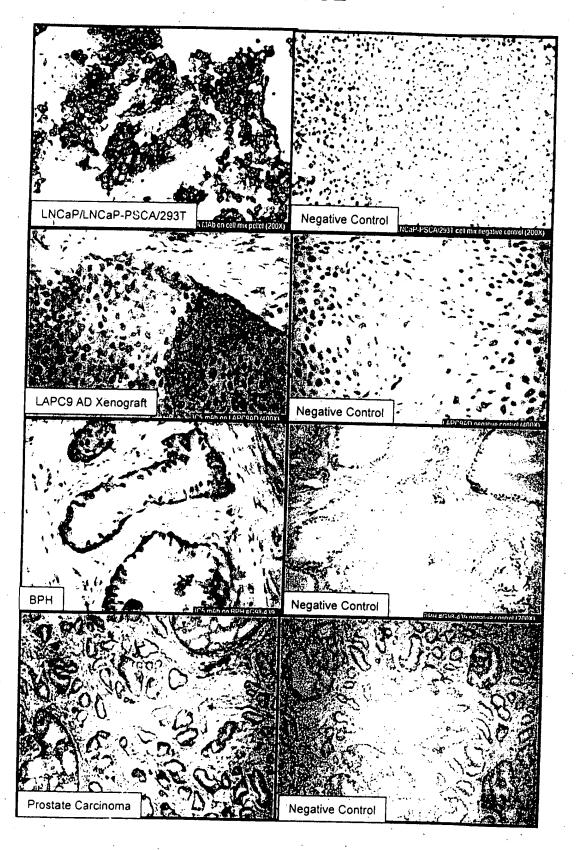
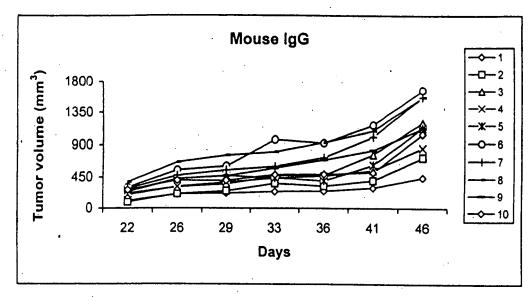
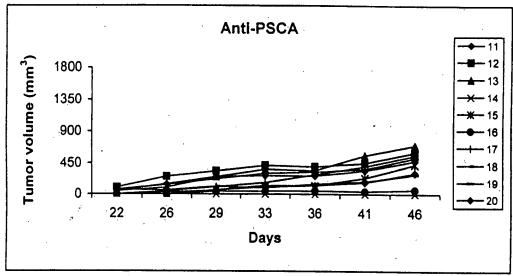
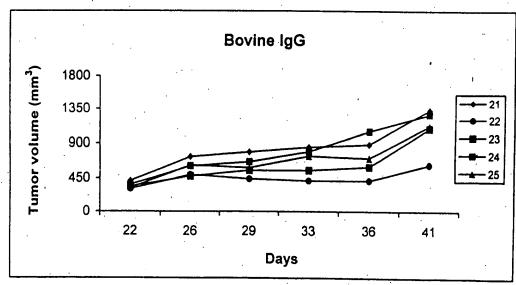
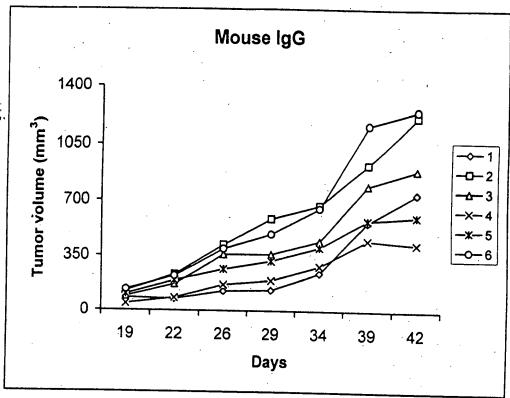


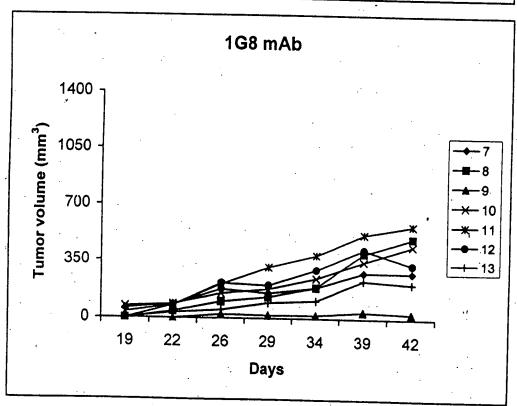
FIG. 53

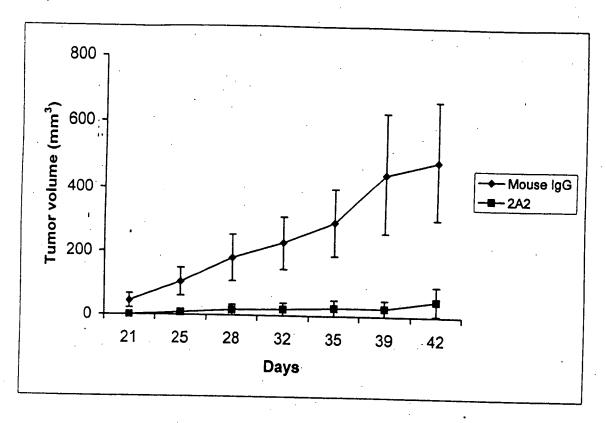


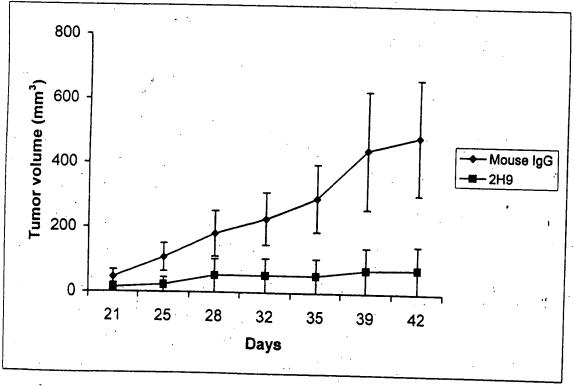


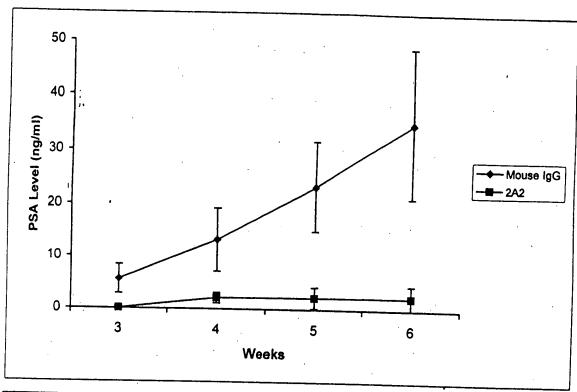


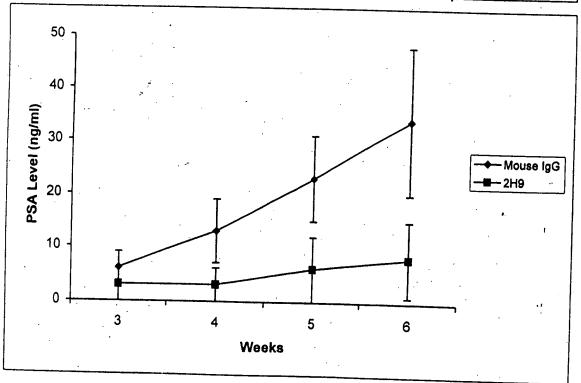




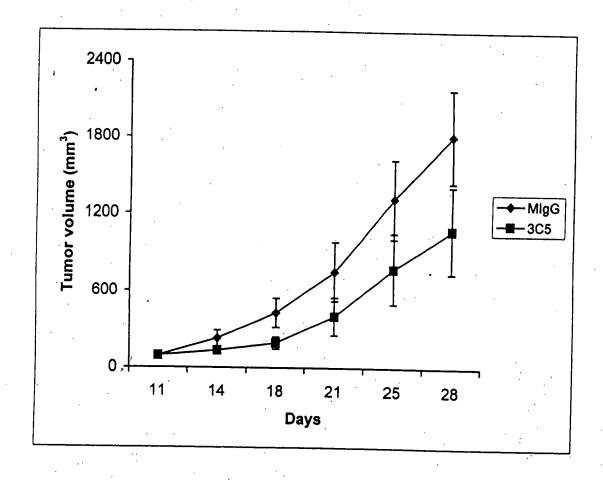












### FIG. 58

TGC	TTC	TTC	CTC	GAT(	GGC!	AGTO	GT?	CATA	\GG <i>I</i>	AGTO	'AA'	TTC	AGAC	GTT	CAC	CTC	CAC	ነ የ	TCT	60
С	F	F	L	М	A	V	V	I	G	Ý	N	s	E		Q	L	Q	Q	S	
GGG	CCA	GA Á	. ריידיים	ייבייי	<u> </u>	ירים וחיב מיים וחיב	\ccc	ימממ	מיטימי	.cmc	13 3 <i>C</i>	mme								
200	N N	.טבער	.C I I	. 610	JOAS	7 1 CF	2002	-	. I C.P.	IG I'C	AAC	TTC	FICC	TGC	CACA					120
. G	A	E	ή	. V	R	S	G	А	S	V	K	. L	S	С	T	A	S	G	<u>F</u>	40
		1	- ÇI	R1		<del></del>								•	٠				TGG	
AAC	ATT	AAA	GAC	CTAC	CTAI	'ATA	CAC	TGG	GTG	AAT	'CAG	AGG	CCI	'GAC	CAG	GGC	CTG	GAG	TGG	180
_N_	_I.	K	D	<u>Y</u>	<u>Y</u>	I	H	W	V	N	Q	R	P	D		G			W	60
					·					CDR	2				*					
ATT	GGA	tgg	ATT	'GA'	rcci	'GAG	AAT	'GGT	GAC	ACT	GAA	لىنىن	יכידר	יככנ	א מ	יייים	CAC	CCA	AAG	240
I	G	W_	I	D	Р	_ E_	N	G	D	Т	Е	F	V	P	_K	F	O	G G	AAG K	80
																			•••	00
GCC.	ACT.	ATG.	ACT	'GCA	AGAC	'ATT	ттс	TCC	AAC	ል ሮ ል	GCC	ጥልጥ	יריזיכי	CNC	ירייר	אממ	אממ	ama.	ACA	
Α	Т	М	т	A	מ	T	F	S	N	·Ψ	Δ	v	1	unc u						
-	_	••	•	-,-	-	•	•		14	•	А	1	יי		ħ	S	5	. ط	<b>T</b> .	100
					•						<u>ر</u> ر	DR3	$\neg$							
TÇT	GAA(	GAC.	ACT	'GCC	GTC	TAT	TAC	TGT.	AAA	ACG	GGG	GGT	TTC	TĠG	GGC	CAA	GGG.	ACT	CTG	360
S	Ε	D	T	A	V	Y	Y	С	K	T	<u>G</u>	G	_F	W	G		G	T	L	120
				<b>4</b> 5														٠		
GTC	ACTO	GTC'	ГСТ	GCA	GCC	AAA	ACG.	ACA	CCC	CCA'	TCT(	GTC	ፐልጥ		ርጥር					
V		V	S.		Α				P		s	v	Y	P	L					

CTGGCC L A

### FIG. 59

TTC	GTA	AGC	AAC	AGC	CTC	AGA:	rgro	CAC	CTC	CCA	GGT(	CAA	ACTO	CAC	3CA2	ACCI	GGC	TCI	GĀA	60
L	V	<b>A</b> ;.	Т	Α	S	D		Н	S			Q		Q	Q	P	G	S	E	20
CTC	<del>ነር</del> ጥር	:AGC	ברריו	iggz	א מיי	יירי <i>ז</i>	ኒርጥር	ממני	יריתים	<u>ደ</u> ሞረረ	מיינים (	ግአ አረ	יממיי	יחיים	naac	· ·		. m.m. c	TCC	
L	v	R	P	. G	T T	S	v	K	T,	S	C	-AAC K	A A	S						
_	•		•	•	•	_	•	10		5	C	10	A	3	<u> </u>	Y_				40
		•																.CL	R1	
AGC	TAC	TGO	ATG	CAC	TGG	GTO	AAG	CAG	AGG	CCI	GGA	CAA	GGC	CTT	GAG	тсс	יייע	יככא	AAT	180
_S_			M		W		K				G		G		E	W	T	.G	N	60
								-				-	_		_	••	•	•	<b>A</b> I	00
ATT	GAC	CCI	GGT	`AGI	GGT	'TAC	ACT	AAC	TAC	GCI	GAG	AAC	CTC	AAG	ACC	AAG	GCC	ACA	CTG	240
I			G					_N_				N				K	Α	T	L	80
					CDR	.2														
															,					
ACT												CTC	AGC	AGC	CTG	ACA	TCT	GAG	GAC	300
T	V	D	T	S.	S	S	T	Α	Y	M	Q	L	S	S	Ł	, <b>T</b>	s	E	D	100
			•																	
~~~																				
							AGC												TGG	360
S	A	V.	Y	Y	С	T	S	R_	S	<u> </u>	_M_	_I_				F	_A_	Y	W	120
				ا. ح	:								CD	R3						
acc	~ n n	~~~	л <i>С</i> Т	ama	ama	3 (JIII)	amai	TI (CC)	~~~	~~~										
G		J G	T T	CIG	GIC.	ACT T	GTC' V										GTC		CCA	420
G	¥	G	1	٠.	V	1	V	S	A	Α.	T	T	Т	A	۰P	S	V	Y	P	160

### FIG. 60

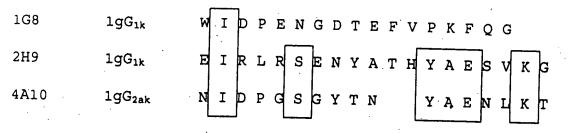
AA	TGA	CTT	CGG	GTT	'GAG	CTG	GGT	TTT	TAT	ידאיי	ጥርጥ	شاش	ውውው	אאא	አጣጣ	000			TGA	
N	D	F	G	L	S	W	v	, E	<del>-</del>	Ι	7,7		1 1 1.						TGA	4 60
		;	. •	_		•	•				V		با	K	G	V	R	: s	Ε	20
GT	GAG	GCT'	ΓGA	GGA	GTC'	TGG.	AGG	AGG	СТС	GGT	GCA	א ררי	ייכרי	700	א מווע	~~ ~			CTCC	
V	R	L	E	E	S	· G	G	G	W	V	O C 1 1	ת ח	7.00						CTCC	120
		1.				J	J	J	. "	v	Q	P	G	G	S	M	K	L	S	40
TG	TGT	AGC	TC	rgg/	ATT	TAC'	rtt	CAG	TAA	TTA	CTG	GATO	BACT	ኮጥርረ	ጋር <b>ታ</b> ጥ	רכ <u>י</u>	רכא.	CTLC	ICCA	
С	V	A	S	G	F	·T	F	s	N	Y	W	M	т	W						
÷	4			•					DR1					**	V	R	. Q	S	P	60
GAG	GAAC	GGG	CTT	rga(	GTG	GGT?	rgc'	rga.	ААТ	TCG	TTC	SAGZ	\ \ '	י ע גאיז ז ע גאיז	יממנ	י דירי אי	ሞረረ	3 A C 1	ACAT	
Ε	K	G	L	E	W	V	Α	E	I	R	Ţ,	· R	9	. O. L	NT	A T T T	160	AACA	ACAT	240
				,			*							<u>Fi</u> _	CDI		A	T_	<u> </u>	80
TAT	rgcg	GAG	TCI	GTG	AAA	\GG@	AAA	\ \ TT(	CACC	ገልጥር	מייריי	ልርአ	CAT	יר א ידי	IM.C.	33.01	~			300 -
<u>Y</u>	. A	_E_	S	V	K	G	K	म	T	T	· · · ·	R	D							300
		·					••	•	•	1	3		ט	D	S	R	S	R	L	100
TAC	CTG	CAA	ATG	AAC	'AAC	TTA	AGA	CCI	GAZ	AGAC	аст	GGA	ል ጥጥ	יוט ער יוטי	יייי א ריייי	יחימו	n		'GGŤ	
Y	L	Q	М	N	N	L	R	Þ	E	D	s	C	T						GGT	360
					ş1 30			•	_	,	J	G	1	Y	Y	C	T	D	<u>Ģ</u>	120
CTG	GGA	CGA(	CCT	AAC	TGG	GGC	CAA	.GGG	ACT	'CTG	ርጥር	א כיתי	صبت	<b>т</b> Ст.	~~»	~~			ACA	
<u> </u>	_G_	R	P	_N	W	G	0	G	T	T.	V V		V							420.
	C	DR3		_	•				. <b>-</b>	-			V	3	A	A	K	Т	Т	140
CCC	CCA:	rcto	GTC'	TAT	CCA	CTG	GCC	CCT	TGT	GTA	•									
P	P	S	V	Y	P	_	Α	p	C	V							•			

### CDR1 Comparisons

		1gG <sub>1k</sub>	Middle	G	F	N	I	K	D	Y	Y	I	Н
2H9		1gG <sub>1k</sub>	N-Term.	G	F	T	F	S	N	Y	W	M	т
4A10	1 .	1gG <sub>2ak</sub>	N-Term.	G	Ÿ	Т	F	s	S	Y	W	M	Н

FIG. 61

### CDR2 Comparisons



### CDR3 Comparisons

1G8	1gG <sub>1k</sub>	G	G	F								٠,
2H9	1gG <sub>1k</sub>	L	G	R	P	N						
4A10	$1gG_{2ak}$	R	S	T	М	I	T	T	G	F	Α	·Y

FIG. 62

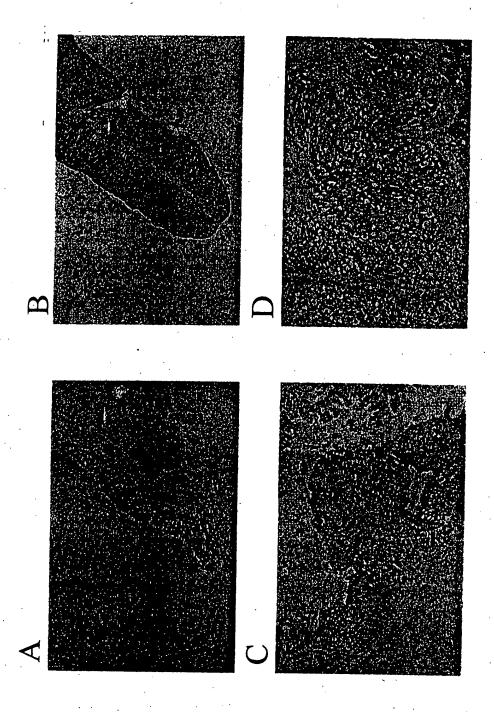
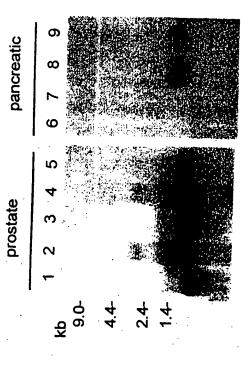
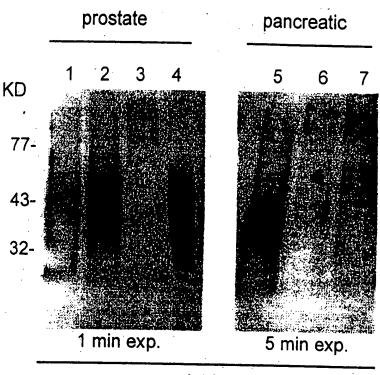


FIG. 63



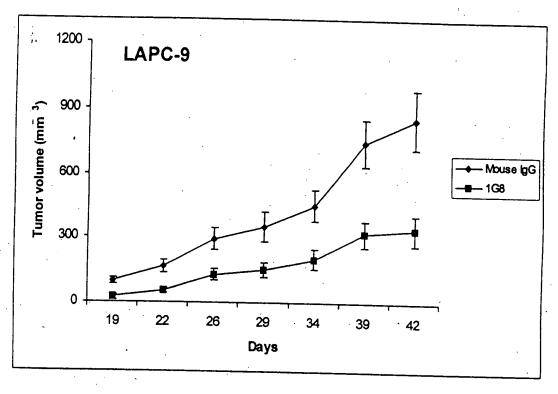
1. Prostate 6. PANC-1 2. LAPC-4 AD 7. BxPC-3 3. LAPC-4 AI 8. HPAC 4. LAPC-9 AD 9. Capan-1 5. LAPC-9 AI

FIG. 64



anti-1G8

- 1. LAPC-4 AD
- 2. LAPC-9 AI
- 3. LNCaP
- 4. LNCaP-PSCA
- 5, HPAC
- 6. Capan-1 7. ASPC-1



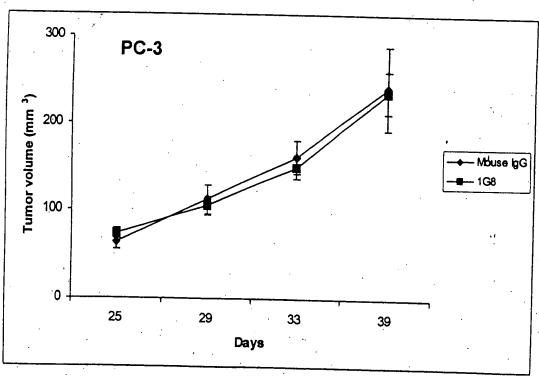
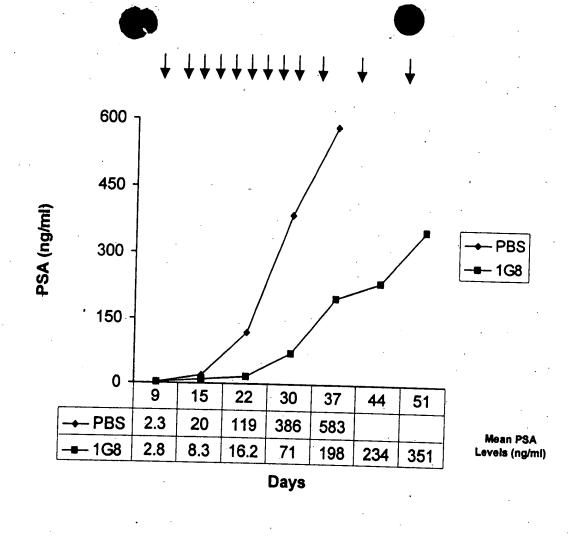


FIGURE 65

B)

A)



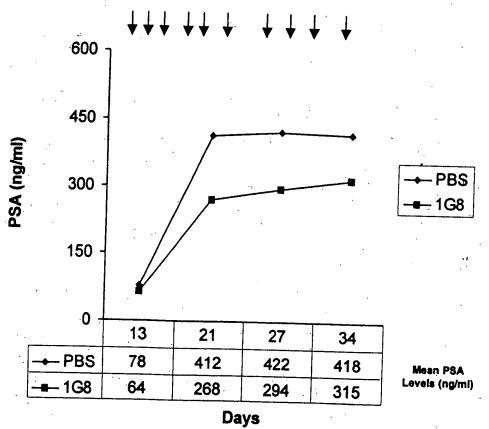
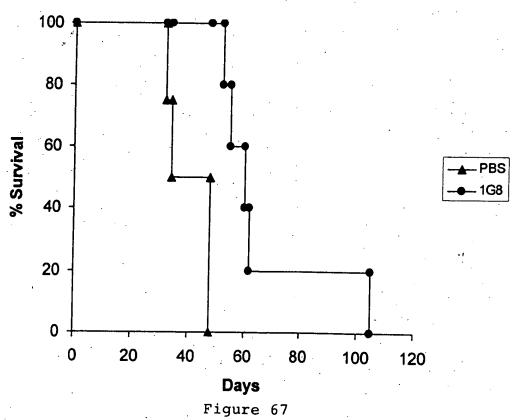
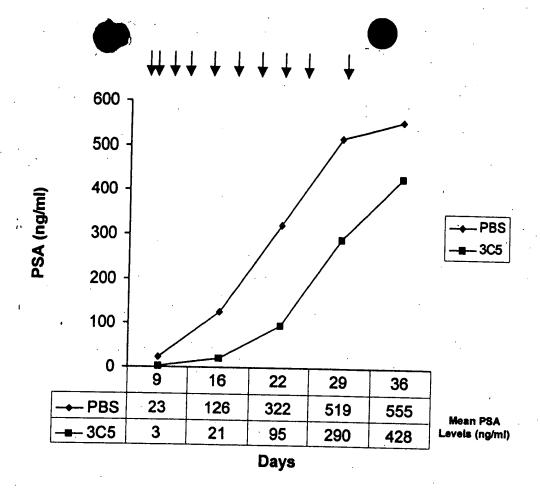


Figure 66

He had been the total that that the same of the term that the term that



B)



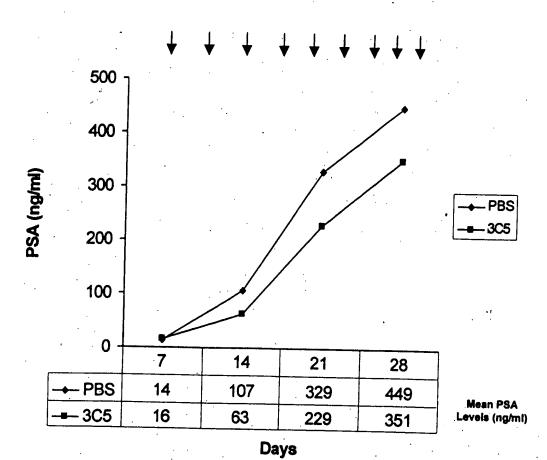
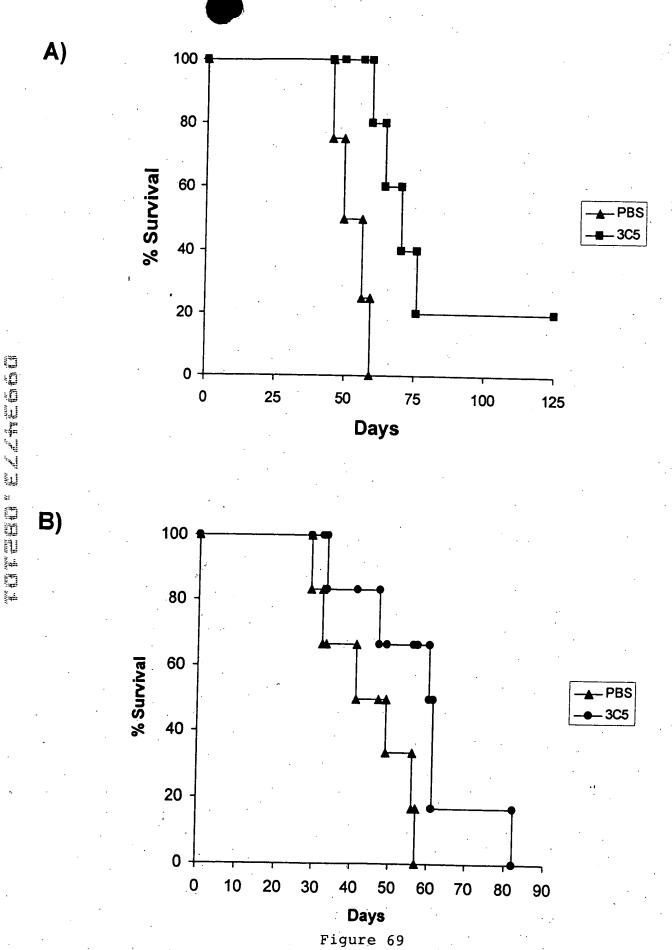


Figure 68



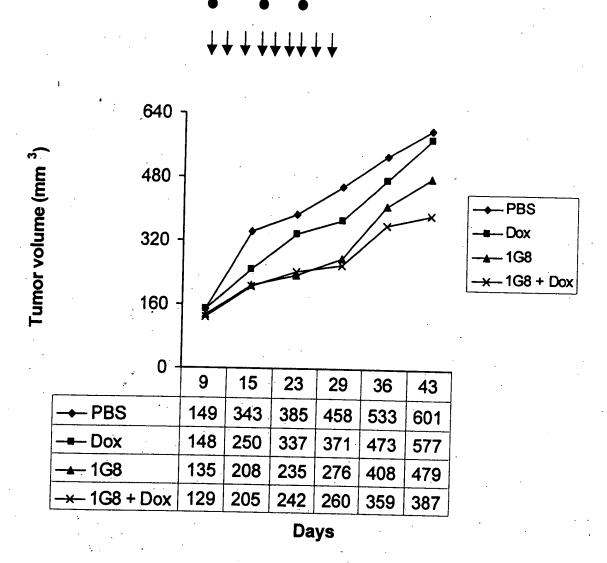
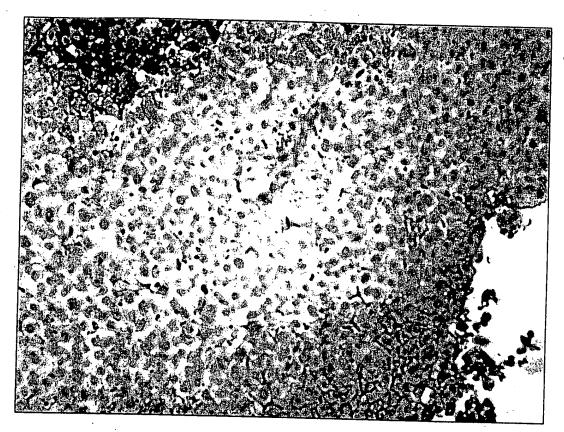


Figure 70

### PSCA 3C5 MAb Localizes within LAPC9AD Xenograft Tissue



3C5 Treated



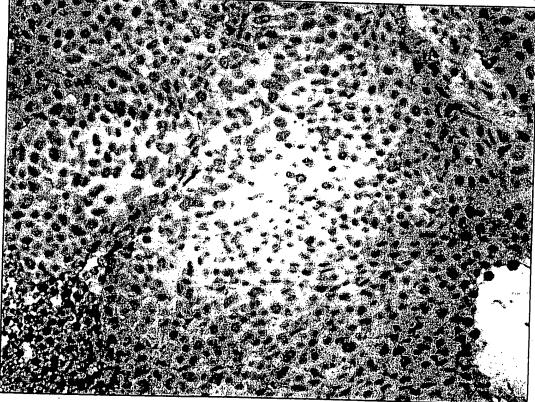
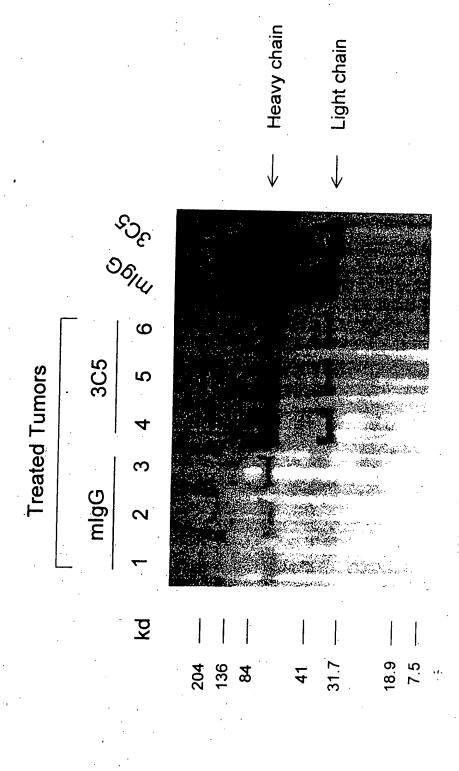


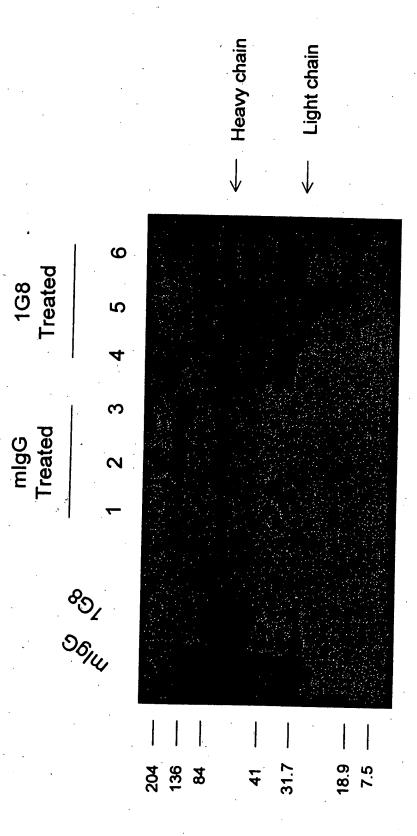
Figure 71

### 3C5 Anti-PSCA MAb is Localized to Established **LAPC-9 Tumors**



Western blot developed with  $\alpha ext{-mlgG/K}$ 

### SPECIFIC TARGETING OF THE 1G8 ANTI-PSCA MAD **TO ESTABLISHED LAPC-9 TUMORS**



- α-MigG Western

Method: Mice bearing established LAPC-9 tumors (>100 mm³) were injected with either mlgG or the anti-PSCA MAb 1G8. Tumors were harvested a week later and made into protein lysates for Western analysis.